## DNA Digestion

**Protocol for DNA Digestion New England Biolabs**

Materials:

- Plasmid or DNA fragment
- Cutsmart EcorI and SpeI (Upstream)
- XbaI and PstI (Downstream)
- PstI and EcorI for the vector
- Buffer Tango
- Thermocycler
- Agarose
- GelRed
- Buffer TAE
- Molecular Marker
- Loading Buffer
- Samples
- Electrophoresis chamber
- Nuclease Free Water (NFW)

Digestion:

1. Add the following volumes to a PCR reaction tube.

| Reagent | Volume/mass |
|---|---|
| Plasmid or DNA Fragment | 30 ng/µl |
| Buffer Tango | 2 µl |
| Upstream Enzyme | 1 µl |
| Downstream Enzyme | 1 µl |
| NFW | Fill up to 20 µl |
| Total | 20 µl |

2. Incubate at 37°C for 15 minutes. Then incubate at 80°C for 20 minutes.
3. Store at -20°C.

Confirmation:

1. Solve 1,5g of Agarose in 100 mL of buffer TAE 1X by heating the mix in the microwave. Be careful to prevent bubbles.
2. Add 1 µl of GelRed
3. Pour in the electrophoresis chamber and let the gel solidify.
4. Draw your run map.
5. When the gel is ready remove the well comb and charge the samples according to the run map.
    a. 5 µl of sample
    b. 2 µl of loading buffer
6. Add to the first well 5 µl of the molecular marker.
7. Run the electrophoresis for 45 minutes at 90 V.